

SHORT COMMUNICATION

Bart Janssen · Julian Sampson · Mieke van der Est
 Wout Deelen · Senno Verhoef · Ian Daniels
 Arjenne Hesseling · Phillip Brook-Carter · Mark Nellist
 Dick Lindhout · Lodewijk Sandkuijl · Dicky Halley

Refined localization of TSC1 by combined analysis of 9q34 and 16p13 data in 14 tuberous sclerosis families

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Abstract Tuberous sclerosis (TSC) is a heterogeneous trait. Since 1990, linkage studies have yielded putative TSC loci on chromosomes 9, 11, 12 and 16. Our current analysis, performed on 14 Dutch and British families, reveals only evidence for loci on chromosome 9q34 (TSC1) and chromosome 16p13 (TSC2). We have found no indication for a third locus for TSC, linked or unlinked to either of these chromosomal regions. The majority of our families shows linkage to chromosome 9. We have refined the candidate region for TSC1 to a region of approximately 5 cM between ABL and ABO.

Introduction

Tuberous sclerosis (TSC) is an autosomal dominant disorder, characterized by hamartomas that may affect numerous organ systems (for a review, see Gomez 1991). Positional cloning has been hampered by locus heterogeneity. Linkage was initially reported to markers on chromosome 9q34 (Fryer et al. 1987). However, these findings were subsequently disputed, until significance for locus heterogeneity was demonstrated and the existence of a chromosome-9-linked locus (TSC1) was confirmed (Haines et al. 1991 a, b; Janssen et al. 1991; Northrup et al. 1992).

During the period 1990–1992, linkage studies in TSC were dominated by two main strategies: the development of more efficient methods for heterogeneity analysis and the search for other loci responsible for the disease. Sev-

eral methods for linkage analysis under heterogeneity have been used. The HOMOG programs (Ott 1991), implementing the admixture test (A-test), have been used successfully by several research groups (Haines et al. 1991 a, b; Northrup et al. 1992). A more advanced application of the A-test, which we have designated the “imaginary chromosome approach” (ICA) (Janssen et al. 1990), involves the synchronous analysis of multipoint linkage data from multiple candidate regions. The A-test based approaches are relatively powerful and provide an efficient tool for the assignment of the gene defect, in each family, to one of the various loci (Janssen et al. 1992). Alternative transparent techniques that are not based on the A-test have supported the findings obtained with A-test based approaches (Povey et al. 1991).

Additional chromosomal locations for TSC have been sought since the demonstration of locus heterogeneity. A second locus was provisionally assigned to chromosome 11 (Smith et al. 1990) but subsequent heterogeneity analyses have failed to position a locus within the predicted region on 11q14–23 (Haines et al. 1991 a, b; Janssen et al. 1991; Povey et al. 1991) or have attained marginal significance levels (Janssen et al. 1990). A de novo translocation t(3;12) in a TSC patient led to the provisional assignment of a third locus to chromosome 12, through linkage analysis in 15 German families (Fahsold et al. 1991). However, a large collaborative heterogeneity analysis involving data from all three regions 9q34, 11q14–23 and 12q23.3 provided no evidence for either a locus on chromosome 11 or a locus on chromosome 12 (Sampson et al. 1992). In 1992, Kandt et al. reported linkage to markers on the tip of chromosome 16p in five large non-chromosome-9-linked TSC families. Apart from the incontrovertible evidence for a locus on 16p13.3, the authors also showed a clear lack of evidence for loci on chromosome 11 or 12 in these families. However, since only non-chromosome-9-linked families were studied, no comment on the importance, or even the existence, of a locus on 9q34 could be made. We planned a heterogeneity analysis, methodologically similar to previous studies (Sampson et al. 1992) and utilizing data from 9q34 and 16p13.3, with

B. Janssen · M. van der Est · W. Deelen · S. Verhoef
 A. Hesseling · D. Lindhout · L. Sanjuijl · D. Halley
 MGC Department of Clinical Genetics,
 Erasmus University Rotterdam
 and Academic Hospital Rotterdam, Rotterdam, The Netherlands

J. Sampson · I. Daniels · P. Brook-Carter · H. Nellist
 Institute of Medical Genetics, University of Wales College of
 Medicine, Cardiff, Wales, UK

D. Halley (✉)
 Department of Clinical Genetics, Dr. Molewaterplein 50, 3015
 GE Rotterdam, The Netherlands

Table 1 Lod scores and posterior probabilities (HOMOG2) for individual families

Family	Maximum lod score		Lod score at		Posterior probability of being 9-linked
	Chromosome 9	Chromosome 16	D9S64	D16S291	
4079	0.20	0.86	-1.20	0.86	0.015
4210	0.00	1.14	-3.79	1.13	0.000
4077	1.27	0.26	1.27	-0.41	0.989
4219	1.81	0.00	1.81	-1.74	1.000
4221	0.83	0.05	0.83	-0.27	0.959
4222	0.00	0.00	-0.11	-0.11	0.649
4264	0.72	0.03	0.72	-0.69	0.979
1003	0.00	0.14	-0.59	0.04	0.300
1004	0.00	0.88	-3.32	0.88	0.000
1007	0.00	0.00	-0.15	-0.12	0.636
1011	0.00	0.05	-0.22	-0.41	0.745
1012	0.81	0.00	0.80	-0.26	0.955
1015	0.48	0.00	0.05	-0.52	0.876
1013	3.90	0.00	3.90	-5.43	1.000
Total			0.01	-7.05	

the aims of gaining a better insight into the TSC heterogeneity problem and of achieving more precise map positions for each locus.

Materials and methods

We selected 14 large families from a mixed group of 24 nuclear families and extended pedigrees from Cardiff and Rotterdam. Previously, simulation studies had been performed in order to determine the power of each family (Janssen et al. 1992). Families that never showed two or more informative meioses were left out of the present analysis, because this type of family cannot contribute significantly, unless an extremely large number of families is available. Of the 14 selected families, 13 had previously been included in other analyses. The only changes with regard to family structure or affection status was the extension of family 1013 and the clarification of previously unknown statuses in this family. One new five-generation family (family 4210) has been added. We used the same genetic parameters (corresponding to a penetrance of 95% and a phenocopy rate of 2%) as in our previous study (Sampson et al. 1992). We typed markers at ABL (dinucleotide repeat), D9S64 (dinucleotide repeat), ABO and D9S10 (MCT136) on chromosome 9. Inter-marker distances (2, 3 and 2 cM, respectively) were taken from the report of the Second Chromosome 9 Workshop (Kwiatkowski et al. 1993). At ABO, the serological typing was utilized, unless molecular typing of the O-allele had been performed. In family 1013, D9S10 was uninformative and therefore replaced by the dinucleotide repeat D9S66, which maps on the same cosmid. We typed the markers D16S85 (3'HVR), D16S259 (pGGG1) and the dinucleotide repeats D16S291 (16AC2.5) and D16S283 (SM7) on chromosome 16. Inter-marker distances (6, 1 and 1 cM, respectively) were taken from Germino et al. (1992) or were inferred from physical distances. On both chromosomes, lod scores were calculated at intervals of 1 cM. Outside the inter-marker regions, lod scores were calculated at several positions from 0%–50% recombination. Heterogeneity was studied using data from chromosomes 9 and 16 synchronously by ICA, allowing for two linked loci on the combined chromosomes, or three loci, one of which is unlinked. The data were analysed by the programs HOMOG2 and POINT4 from the HOMOG package (Ott 1991).

Results and discussion

Assuming homogeneity, we obtained a maximum cumulative lod score $Z_{(\alpha=1)\max}$ of 4.14 at 10% recombination ($\Theta = 0.1$) from ABL (Table 1, Fig. 1). At each map position (X_1), we calculated the maximum lod score $Z_{(\alpha, X_1, X_2)\max}$ by optimizing α (proportion of linked families) and the position of the other locus (X_2). On chromosome 9, the lod score peaked at D9S64 ($Z_{(\alpha, X_1, X_2)\max} = 8.92$, $\alpha = 0.65$). By comparison with $Z_{(\alpha=1)\max}$, we found an odds ratio of $6.0 \cdot 10^4:1$ in favour of heterogeneity. Still assuming heterogeneity, we calculated the lod score for TSC1 being unlinked to chromosome 9. This position is associated with a $Z_{(\alpha, X_1 = \infty, X_2)\max} = 0.73$, with $\alpha = 0.72$ and the remainder located at D16S291. ($Z_{(\alpha, X_1 = \infty, X_2)}$ may be unequal to 0 if $\alpha \neq 1$). A comparison of the two lod scores under heterogeneity revealed substantial evidence for TSC1 being linked to chromosome 9 ($Z = 8.2$) with an odds ratio of $1.5 \cdot 10^8:1$. To delineate the area further, a 90% confidence interval was constructed, including all locations with a lod score exceeding $Z_{(\alpha, X_1, X_2)\max} - 1$. ABL and ABO do not lie within this confidence interval and are therefore likely to be flanking markers encompassing a TSC1 region of 5 cM.

On chromosome 16 (under the assumption of homogeneity), we obtained a maximum lod score $Z_{(\alpha=1)\max}$ of 0.52. Under heterogeneity, the locus mapped at D16S291, with $\alpha = 0.35$ and TSC1 placed at D9S64 (Fig. 2) ($Z_{(\alpha, X_1, X_2)\max} = 8.92$ as discussed above). The unlinked position was associated with a $Z_{(\alpha, X_1, X_2 = \infty)\max}$ of 6.52 at $\alpha = 0.43$ and TSC1 placed at D9S64. This results in an odds ratio of $2.5 \cdot 10^2:1$ ($Z = 2.4$) in favour of linkage of "TSC2" to chromosome 16. This result, although not independently significant according to the stringent guidelines that we have proposed elsewhere (Janssen et al. 1992) provides a clear confirmation of Kandt's finding (Kandt et al. 1992), since none of our families were in his

Fig. 1 Results of the multipoint analysis of TSC with markers on chromosome 9. The lower line (■) indicates the cumulative lod score under homogeneity. The upper line (■) indicates the lod score under heterogeneity ($Z_{(\alpha, X_1, X_2)}$), as derived from the POINT4 program. At each position, $Z_{(\alpha, X_1, X_2)}$ was calculated by optimizing α and the position of TSC2. Odds for heterogeneity and linkage are depicted with broken arrows

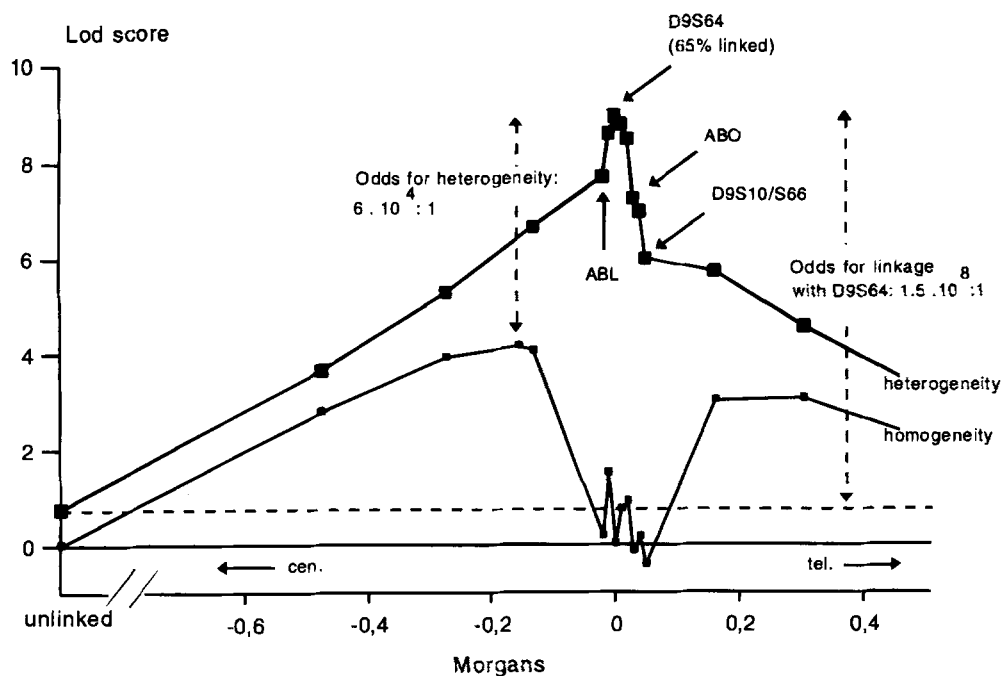
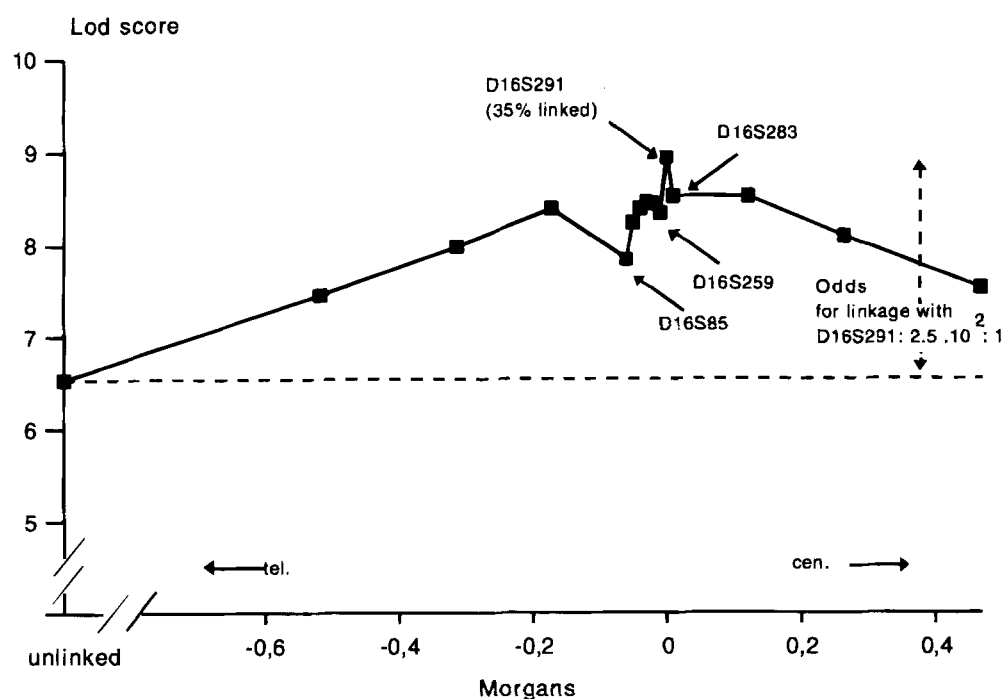


Fig. 2 Results of multipoint analysis of TSC with markers on chromosome 16 (explanation as in Fig. 1). Since the lod score under homogeneity peaked at 0.52, these lod scores are not shown



study. Because of the limited amount of information in the chromosome-16-linked families, we could not define a narrow confidence interval for TSC2. The ratio of large and small families was about the same for both the TSC1 and the TSC2 groups of families.

In order to examine the possibility of a third trait-causing locus, we repeated the analysis. Instead of two α -values, three were introduced, where α_1 and α_2 are as before and where α_3 denotes the proportion of families linked to neither region. A maximum lod score was obtained if $\alpha_3 = 0\%$; thus, this data set yields no evidence for a third locus.

Our results demonstrate the usefulness of ICA. In order to avoid systematic bias, we have chosen to evaluate the lod scores under heterogeneity directly, rather than selecting families by their posterior probability of being linked to one of the regions, followed by an evaluation of their $Z_{(\alpha=1)}$ values, as other authors have done (Haines et al. 1992a, b; Northrup et al. 1992). If we had used such a method, the (inflated) lod score in favour of linkage to 16p13.3 would have been 2.9 instead of 2.4.

During the course of this study, the TSC2 gene on chromosome 16 has been isolated (European Chromosome 16 TSC Consortium 1993). The gene maps less than

200 kb distal of D16S291. In one of the families (family 4079; posterior probability of being chromosome-16-linked = 0.985), a reduced level of TSC2 transcript was demonstrated in all affected family members (European Chromosome 16 TSC Consortium 1993), thus confirming the involvement of TSC2 in this family. The excellent agreement between the statistical and the molecular localization of TSC2 is encouraging for a targeted search for TSC1 in the currently favoured area on chromosome 9. Molecular diagnosis should soon be feasible in large families.

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